REMARKS

Claims 87-111 are pending in the case. These claims have been cancelled without prejudice and new claims 112-194 have been added.

Objection to the Drawings

The drawings have been objected to. In response, Applicant requests that the filing of formal drawings be delayed until there is allowable subject matter.

Specification/Informalities

The occurrence of hyperlinks within the specification has been objected to. In response, Applicant has amended the offending paragraphs to deletes such hyperlinks and to replace these with simple corresponding internet addresses.

The title of the application has been objected to as non-descriptive of the claimed invention. In response, Applicant has cancelled the title and replaced this with a new title following the general suggestion of the Examiner.

The description of Figure 3 has been objected to as containing references to Figures 3A – 3E whereas only a single Figure 3 is depicted. Figure 3 as filed contains lowercase letters at the left of the figure that serve to identify different portions thereof. In response to the objection, Applicants have amended the description, as well as paragraphs throughout the application that refer to portions of Figure 3, so as to indicate the lowercase letter portion of the figure rather than references to what might be misconstrued as separate figures. Thus, no new matter has been added.

New Claim Support

New claims 112-164 have been added to the application. These claims are supported throughout the application by the scope and spirit of the disclosure as well as the knowledge and ability of those skilled in the art who will read and comprehend the application. These claims are more specifically supported as follows:

Claim 112 and 113 are supported in the application at pages 61, 63-65, page 14, lines 9-13, page 15, lines 10-14 and at page 8, lines 9-16. In addition, claim 112 tracks cancelled claim 87 and finds similar support.

In addition, claims are supported as follows:

Claim 114-117 (page 62, lines 12-15, page 14, lines 9-13 and page 8, lines 9-14); use of phospholipid in claim 14 is specifically supported at page 71, line 8 to page 72, line 3;

Claims 118-123 (page 71, line 8 to page 72, line 3)

Claim 126 (page 72, lines 6-12, page 73, line 22 to page 76, line 3)

Claim 127 (page 9, lines 10-12)

Claims 128-130 (page 14, lines 9-14)

Claims 135-138 (page 14, lines 9-13, page 57, lines 24-26 and page 70, line 14 to page 71, line 5)

Claims 139-142 (page 70, lines 1-11)

Claims 143 and 148 – 160 (page 58, lines 1-6, page 66, lines 10-22, page 72, line 15 to page 73, line 7)

Claim 144 (page 2, lines 21-26)

Claims 145-146 (page 72, lines 6-12 and page 72, line 15 to page 73, line 7)

Claim 147 (page 71, line 8 – page 72, line 3)

Claims 161-165 (page 58, lines 7-12, and page 66, line 23 to page 67, line 5)

Claim 166-168 (page 15, line 12)

Claims 169-172 (page 58, lines 13-14)

Claim 173 (page 5, lines 24-25)

Claims 174-175 (pages 5, lines 7-23, and page 57, line 17-24)

Claims 176-178 (page 64, line 26 and pages 18-20, page 57, lines 17-24, and page 69, lines 1-9)

Claims 179-180 (page 65)

Claim 181 (page 5)

Claims 182-183 (page 72, lines 5-12)

Claims 184-188 (page 15, line 10-24, and page 72, lines 6-12)

Claims 189-194 (page 59, lines 19-23 and page 59, line 26 to page 60, line 13)

In addition, claims 124, 149 and 193 recite use of fibroblasts. Such use is supported throughout the application and more specifically at page 3, line 26 to page 4, line 2, at page 16, line 25 to page 17, line 3, at page 21, lines 6 – 10 and lines 19 – 26, at page 22, line 25 to page 23, line 5, at page 24, lines 1-3, at page 27, lines 9 – 10, and at page 32, line 26 to page 34, line 11.

Claims 125, 150 and 194 recite use of macrophages. Support for such use is found throughout the application but more specifically at page 24, lines 4 – 9 (i.e., histiocytes) and at page 27, lines 9 – 10 (i.e., monocytes).

Claim Objections

Claims have been objected to for use of abbreviations without first reciting the entire phrase. The new claims remove this problem by first reciting the term in entirety and only thereafter using the abbreviations. In addition, objections based on grammar have also been corrected in the new claims set.

Rejection Under 35 U.S.C. §112

Claims 87-111, added by Preliminary Amendment, have been rejected under 35 U.S.C. 112, first and second paragraphs, on a number of grounds.

New claims 112-194 are similar to and track the claims previously added by Preliminary Amendment but are believed to better reflect the claimed invention as well as to respond to the basis for rejection contained in the Office Action. As such, these new claims are supported throughout the specification and at the locations previously cited in the Preliminary Amendment filed on 9 November 2000.

Claims 87, 88, 90-94 and 96-111 were rejected under section 112, second paragraph, as being indefinite.

Claims 88 (and dependent claims 100-108 and 111) were rejected as unclear and confusing for use of the term "constituent of an HDL particle." While Applicant does not concede that this language is unclear or confusing to those skilled in the art, these claims have been cancelled and new claims added in their stead. Thus, new claims 116-117 recite use of cholesterol that is part of HDL cholesterol or which is part of a fragment of HDL cholesterol wherein said fragment is sufficient to bind the ABC1 polypeptide.

New Claim 112 replaces cancelled claim 87 and is supported at pages 61 and 63-65 of the application. New claims 116 and 117 depend from claim 112 and recite use of HDL-cholesterol or a fragment of HDL-cholesterol wherein said fragment is sufficient to bind hABC1 polypeptide. Thus, it is clear from these new claims what is being used as a ligand for the hABC1 polypeptide of the claims. Support is found at page 10, lines 12-16, and page 61, lines 13-18.

Claim 87, 88, 95 and 96 (and claims dependent thereon) were rejected as confusing because they do not clearly recite the result that leads to a conclusion of modulation. In response, Applicants have cancelled these claims and added new claims in their place.

New claim 112 is believed to clearly recite a comparison between the activity of the polypeptide when a test compound is present versus when it is not present. This

claim is supported in the application at pages 61, lines 13-18 and lines 19-25, page 62, lines 12-15, page 63, lines 23-26, and at page 14, lines 9-13.

Claims 87-91, 93-95, 97-103, and 105-11 have been rejected under section 112, first paragraph, as not being supported in the specification in such manner as to convey to those in the art that the applicants were in possession of the claimed invention at the time the application was filed. Specifically, this rejection is based on use of the term ABC polypeptide whereas the specification teaches use of a human ABC polypeptide. In response, these claims have been cancelled and new claims added. Said new claims specifically recite use of a mammalian polypeptide for the assays of the invention as well as the use of compounds identified through said assays for the identification of therapeutic agents useful in reducing triglyceride and cholesterol levels in animals.

Further, Applicants believe that use of ABC1 from polypeptides is clearly supported in the application as filed because Applicants show comparisons between human and mouse sequences and comparisons of these to, for example, *Caenorhabditis elegans*, as a means of showing conserved regions. Thus, assays employing mouse ABC1 are supported at page 9, lines 10-12, of the application as filed. Comparison of maouse and human sequences with non-mammalian sequences are shown in Figure 4B, and the description of Figure 4B at page 18, lines 7-13, in Figure 5B and the description of Figure 5B at page 19, lines 6-11, in Figure 6B and the description of Figure 6B at page 20, lines 3-8, and in Figure 6E and the description of Figure 6E at page 20, lines 22-26. These portions of the application also reveal the location of critical mutations occurring within these sequences. The mouse and human sequences are also compared to that of WHAM chickens in Figure 15 and the description of Figure 15 at page 23, lines 9-10 of the application as filed. In addition, as disclosed in the application at page 41, lines 12-14, the mouse sequence has Accession number X75926 but lacks the first 60 residues of the human polypeptide and so is not complete. Applicants are the first to identify this problem and the importance of this N-terminal sequence.

In view of said amendment, and the foregoing discussion, Applicants believe that this ground of rejection has been overcome and should be withdrawn and that claims to mammalian, including claims to processes utilizing mouse or human ABC1 polypeptides, should be allowed.

Claims 87-111 have also been rejected under section 112, first paragraph, as not being enabled by the disclosure of the specification because the claims are being read as reciting use of specific assay conditions and ligands as a means of identifying compounds that modulate all activities of the ABC polypeptide using any lipid.

Applicant respectfully traverses this ground of rejection as follows. The claims are directed to a process for identifying a compound that modulates ABC polypeptide activity. The claims are directed toward identifying ABC modulators and do not reasonably imply that any and all activities of ABC polypeptide are affected by the agents identified using these assays. For example, new claim 112 is directed to identifying a compound that modulates ABC polypeptide activity and any clear reading of the assay procedure recited in the claim makes clear that activities affected by the presence or absence of the compound are all that is being claimed. Certainly, any compound that affects the recited activity is clearly a modulator of ABC activity and that is all the claim requires.

In addition, Applicants have cancelled claim 87 and added new claim 112 that specifically recites use of a lipid that binds ABC1, which lipids are either already known to those skilled in the art or can readily be determined without undue experimentation since procedures for determining such binding are well known and are described in the application. In view of the foregoing, Applicants believe that this ground of rejection has been overcome and that the new claims are in condition for allowance.

Claims 87-111 have also been rejected as being overly broad. In response, Applicants have cancelled these claims and added new claims 112-194, which are believed to more clearly recite the claimed invention as disclosed in the application. In particular, new claim 112 recites use of a binding assay of ABC polypeptide and a lipid

that binds ABC polypeptide under conditions promoting said binding as a means of identifying modulating agents. New claims 113-134 depend directly or indirectly from claim 112 and therefore recite the same limitation.

New claims 135-142 recite use of ATP binding or hydrolysis as a means of identifying modulating agents. Methods of measuring ATP binding and/or hydrolysis are well known in the art.

New claims 143-160 recite use of transport assays specifically described in the application at the locations recited above in support of such claims. Because ABC polypeptide is involved in such transport, compounds that affect this activity are modulators of the ABC polypeptide. The use of different components of HDL in claims 145-147 is likewise specifically supported in the application (see claim support provided above).

New claims 176-181 are directed to use of mutant ABC polypeptides as disclosed in the application. Applicants provide the sequence of mammalian ABC1 polyptides and thus the identity of mutated forms is readily determined. In addition, such mutant forms can be readily employed in the assays of the application just as the native ABC polypeptide is used.

New claims 182-188 are directed to assays for compounds that modulate triglyceride and cholesterol levels in animal as disclosed in the application. Because such levels are easily measured by methods known in the art, the use of compounds identified as ABC modulators using the assays of claims 112-181 is readily accomplished.

New claims 189-194 are directed to assays based on the expression of the ABC polypeptide in cells that express it. Because this polypeptide is a surface protein and because antibodies to it can readily be generated based on the polypeptide structure disclosed in the application, such expression is readily measured thereby enabling such a procedure.

In light of the foregoing, Applicant respectfully requests that the Examiner withdraw the current grounds of rejection and allow the pending claims to issue.

In addition, Applicants enclose Form 1449 with copies of two additional references to be considered by the Examiner. WO 98/48784 (publication date 5 November 1998) relates to methods of using ABC blockers to block amyloidosis as a means of dealing with Alzheimer's Disease (see claim 1 therein). WO 00/24390 (publication date 4 May 2000) is similar in scope. Applicants also note that the present application claims an earliest priority date, based on a provisional application, of 15 March 1999.

Applicants have enclosed a Request for an extension of time by 1 month for filing of this response and a check to cover the cost for a large entity. No other fee believed due in filing the above amendment. The Commissioner is requested to charge any additional fees, or credit any refunds, to Deposit Acc't No. 03-0678.

FIRST CLASS CERTIFICATE

I hereby certify that this correspondence is being deposited today with the U.S. Postal Service as First Class Mail in an envelope addressed to:

Commissioner for Patents
Washington, DC 20231

Alan J. Grant 10/19/01

Alan J. Grant, Esq. **Date**

Respectfully submitted,

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AMENDED SPECIFICATION

The paragraph starting at page 17, line 10, has been amended as follows:

~~Figs. 3A - 3E are~~ Figure 3 is a schematic illustration showing a genetic and physical map of 9q31 spanning 35 cM. Lowercase letters a-e on the left delineate different aspects of the maps. ~~Fig. 3A:~~ Fig. 3 at a: YACs from the region of 9q22-34 were identified and a YAC contig spanning this region was constructed. ~~Fig. 3B:~~ Fig. 3 at b: A total of 22 polymorphic CA microsatellite markers were mapped to the contig and used in haplotype analysis in TD-1 and TD-2. ~~Fig 3C:~~ Fig. 3 at c: The mutant haplotypes for probands in TD-1 and –2 indicate a significant region of homozygosity in TD-2, while the proband in TD-1 has 2 different mutant haplotypes. The candidate region can be narrowed to the region of homozygosity for CA markers in proband 2. A critical crossover at D9S1690 in TD-1 (A)* also provides a centromeric boundary for the region containing the gene. Three candidate genes in this region (*ABC1*, *LPA-R* and *RGS-3*) are shown. ~~Fig. 3D:~~ Fig. 3 at d: Meiotic recombinations in the FHA families (A-H) refine the minimal critical region to 1.2 cM between D9S277 and D9S1866. The heterozygosity of the TD-2 proband at D9S127, which ends a continuous region of homozygosity in TD-2, further refines the region to less than 1 cM. This is the region to which ABC1 has been mapped. ~~Fig. 3E:~~ Fig. 3 at e: Isolated YAC DNA and selected markers from the region were used to probe high-density BAC grid filters, selecting BACs which via STS-content mapping produced an 800 Kb contig. Four BACs containing *ABC1* were sequenced using high-throughput methods.

The paragraph at page 28, lines 14-21, has been amended as follows:

Multiple DNA markers were genotyped in the region of 9q31 to which linkage to TD had been described (Rust et al., Nat. Genet. 20, 96-98, 1998). Two point linkage analysis gave a maximal peak LOD score of 6.49 at D9S1832 (Table 1) with significant evidence of linkage to all markers in a ~10 cM interval. Recombination with the most proximal marker, D9S1690 was seen in II-09 in Family TD-1 (A* in ~~Fig. 3D~~ Fig. 3 at d) providing a centromeric boundary for the disease gene. Multipoint linkage analysis of these data did not increase the precision of the positioning of the disease trait locus.

The paragraph at page 28, starting at line 22, has been amended as follows:

A physical map spanning approximately 10 cM in this region was established with the development of a YAC contig (~~Fig. 3A~~ Fig. 3 at a). In addition, 22 other polymorphic multi-allelic markers which spanned this particular region were mapped to the contig (Fig. 3B) and a subset of these were used in construction of a haplotype for further analysis (Figs. 1A and 1B; Table 2). The condensed haplotype in these families is shown in Figs. 1A and 1B.

The paragraph at page 31, lines 3-8 has been amended as follows:

While the family of Dutch decent did not demonstrate any consanguinity, the proband in TD-2 was the offspring of a first-cousin consanguineous marriage (Fig. 1B). We postulated, therefore, that it was most likely that this proband would be homozygous for the mutation while the proband in the Dutch family was likely to be a compound heterozygote. The Dutch proband shows completely different mutation bearing haplotypes, supporting this hypothesis (~~Fig. 3C~~ Fig. 3 at c).

The paragraph at page 31, lines 9-13, has been amended as follows:

The TD-2 proband was homozygous for all markers tested (Fig. 1B) distal to D9S127 but was heterozygous at D9S127 and DNA markers centromeric to it (~~Fig. 3C~~ Fig. 3 at c). This suggested that the gene for TD was likely located to the genomic region telomeric of D9S127 and encompassed by the markers demonstrating homozygosity (~~Fig. 3B~~ Fig. 3 at b).

The paragraph at page 31, starting at line 16, has been amended as follows:

Based on the defect in intracellular cholesterol transport in patients with TD, we reviewed the EST database for genes in this region which might be relevant to playing a role in this process. One gene that we reviewed as a candidate was the lysophosphatidic acid (LPA) receptor (*EDG2*) which mapped near D9S1801 (~~Fig. 3C~~

Fig. 3 at c). This receptor binds LPA and stimulates phospholipase-C (PLC), and is expressed in fibroblasts. It has previously been shown that the coordinate regulation of PLC that is necessary for normal HDL3 mediated cholesterol efflux is impaired in TD (Walter et al., J. Clin. Invest. 98:2315-2323, 1996). Therefore this gene represented an excellent candidate for the TD gene. Detailed assessment of this gene, using Northern blot and RT-PCR and sequencing analysis, revealed no changes segregating with the mutant phenotype in this family, in all likelihood excluding this gene as the cause for TD. Polymorphisms were detected, however, in the RT-PCR product, indicating expression of transcripts from both alleles.

The paragraph at page 32, lines 4-10, has been amended as follows:

The second candidate gene (*RGS3*) encodes a member of a family regulating G protein signaling which could also be involved in influencing cholesterol efflux (Mendez et al., Trans. Assoc. Amer. Phys. 104:48-53, 1991). This gene mapped 0.7 cM telomeric to the LPA-receptor (~~Fig. 3C~~ Fig. 3 at c), and is expressed in fibroblasts. It was assessed by exon-specific amplification, as its genomic organization was published (Chatterjee et al., Genomics 45:429-433, 1997). No significant sequence changes were detected.

The paragraph at page 32, lines 11-25, has been amended as follows:

The *ABC1* transporter gene had previously been mapped to 9q31, but its precise physical location had not been determined (Luciani et al., Genomics 21:150-159, 1994). The *ABC1* gene is a member of the ATP binding cassette transporters which represents a super family of highly conserved proteins involved in membrane transport of diverse substrates including amino acids, peptides, vitamins and steroid hormones (Luciani et al., Genomics 21:150-159, 1994; Dean et al., Curr. Opin. Gen. Dev. 5:779-785, 1995). Primers to the 3' UTR of this gene mapped to YACs spanning D9S306 (887-B2 and 930-D3) compatible with it being a strong candidate for TD. We initiated large scale genomic sequencing of BACs spanning approximately 800 kb around marker D9S306 (BACs 269, 274, 279 and 291) (~~Fig 3E~~ Fig. 3 at e). The *ABC1* gene was revealed encompassing 49 exons and a minimum of 75 Kb of genomic sequence. In view of the potential function of a gene in this family as a cholesterol transporter, its expression in fibroblasts and localization to

the minimal genomic segment underlying TD, we formally assessed ABC1 as a candidate.

The paragraph at page 36, lines 12-18, has been amended as follows:

As described herein, the *ABC1* gene mapped within this interval. The overlapping genetic data strongly suggested that FHA may in fact be allelic to TD. Utilization of sets of genetic data from FHA and TD provided a telomeric boundary at D9S1866 (meiotic recombinant) (~~Fig. 3D~~ Fig. 3 at d) and a centromeric marker at D9S127 based on the homozygosity data of TD-2. This refined the locus to approximately 1 Mb between D9S127 and D9S1866. The *ABC1* gene mapped within this minimal region (~~Fig. 3E~~ Fig. 3 at e).

The paragraph at page 41, lines 15-21, has been amended as follows:

Version 1.7 of ClustalW was used for multiple sequence alignments with BOXSHADE for graphical enhancement (~~http://~~www.isrec.isb-sib.ch:8080/software/BOX_form.html) with the default parameter. A *Caenorhabditis elegans* ABC1 orthologue was identified with BLAST (version 2.08) using CAA1005.1 (see above) as a query, with the default parameter except for doing an organism filter for *C. elegans*. The selected protein sequence has accession version number AAC69223.1 with a score of 375, and an E value of 103.

The paragraph at page 51, lines 8-15, has been amended as follows:

Transmembrane prediction programs indicate 13 transmembrane (TM) regions, the first one being between amino acids 26 and 42 (~~http://~~psort.nibb.ac.jp:8800/psort/helpwww2.html#ealom). The tentative number of TM regions for the threshold 0.5 is 13. (INTEGRAL Likelihood = -7.75 Transmembrane 26-42). The other 12 TM range in value between -0.64 and -12 (full results below). It is therefore very likely that the newly-discovered 60 amino acids contain a TM domain, and that the amino end of ABC1 may be on the opposite side of the membrane than originally thought.

The paragraph at page 59, lines 10-25, has been amended to read as follows:

Human and rodent ABC1 protein can be used as an antigen to raise antibodies, including monoclonal antibodies. Such antibodies will be useful for a wide variety of purposes, including but not limited to functional studies and the development of drug screening assays and diagnostics. Monitoring the influence of agents (e.g., drugs, compounds) on the expression or biological activity of ABC1 can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase ABC1 gene expression, protein levels, or biological activity can be monitored in clinical trails of subjects exhibiting altered ABC1 gene expression, protein levels, or biological activity. Alternatively, the effectiveness of an agent determined by a screening assay to modulate ABC1 gene expression, protein levels, or biological activity can be monitored in clinical ~~trails~~ trials of subjects exhibiting decreased altered gene expression, protein levels, or biological activity. In such clinical trials, the expression or activity of ABC1 and, preferably, other genes that have been implicated in, for example, cardiovascular disease can be used to ascertain the effectiveness of a particular drug.

The paragraph beginning at the top of page 64 of the application has been amended as follows:

ABC1 protein (or a polypeptide fragment thereof or an epitope-tagged form or fragment thereof) is harvested from a suitable source (e.g., from a prokaryotic expression system, eukaryotic cells, a cell-free system, or by immunoprecipitation from ABC1-expressing cells). The ABC1 polypeptide is then bound to a suitable support (e.g., nitrocellulose or an antibody or a metal agarose column in the case of, for example, a his-tagged form of ABC1). Binding to the support is preferably done under conditions that allow proteins associated with ABC1 polypeptide to remain associated with it. Such conditions may include use of buffers that minimize interference with protein-protein interactions. The binding step can be done in the presence and absence of compounds being tested for their ability to interfere with interactions between ABC1 and other molecules. If desired, other proteins (e.g., a cell lysate) are added, and allowed time to associate with the ABC polypeptide. The

immobilized ABC1 polypeptide is then washed to remove proteins or other cell constituents that may be non-specifically associated with it the polypeptide or the support. The immobilized ABC1 polypeptide is then dissociated from its support, and so that proteins bound to it are released (for example, by heating), or, alternatively, associated proteins are released from ABC1 without releasing the ABC1 polypeptide from the support. The released proteins and other cell constituents can be analyzed, for example, by SDS-PAGE gel electrophoresis, Western blotting and detection with specific antibodies, phosphoamino acid analysis, protease digestion, protein sequencing, or isoelectric focusing. Normal and mutant forms of ABC1 can be employed in these assays to gain additional information about which part of ABC1 a given factor is binding to. In addition, when incompletely purified polypeptide is employed, comparison of the normal and ~~muatant~~ mutant forms of the protein can be used to help distinguish true binding proteins.

The paragraph at page 65, lines 16-17, of the application has been amended as follows:

Another assay ~~is~~ includes a Fluorescent Resonance Energy Transfer (FRET) assay. This assay can be performed as follows.

The paragraph at page 74, lines 1-14, has been amended as follows:

This chicken low HDL locus (Y) is Z-linked, or sex-linked. (In birds, females are ZW and males are ZZ). Genetic mapping placed the Y locus on the long arm of the Z chromosome (Bitgood, 1985), proximal to the ID locus (Bitgood, 1988). Examination of current public mapping data for the chicken genome mapping project, ChickMap (maintained by the Roslin Institute; ~~http://~~www.ri.bbsrc.ac.uk/chickmap/ChickMapHomePage.html) showed that a region of synteny with human chromosome 9 lies on the long arm of the chicken Z chromosome (Zq) proximal to the ID locus. Evidence for this region of synteny is the location of the chicken aldolase B locus (ALDOB) within this region. The human ALDOB locus maps to chromosome 9q22.3 (The Genome Database, ~~http://gdb~~www.gdb.org/), not far from the location of human ABC1. This comparison of maps showed that the chicken Zq region near chicken ALDOB and the human 9q region near human ALDOB represent a region of synteny between human and chicken.